



## Research report

Bacterial enzymes effectively digest Alzheimer's  $\beta$ -amyloid peptide

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## ABSTRACT

Aggregated  $\beta$ -amyloid peptides play key roles in the development of Alzheimer's disease, and recent evidence suggests that microbial particles, among others, can facilitate their polymerization. Bacterial enzymes, however, have been proved to be beneficial in degrading pathological fibrillar structures in clinical settings, such as strepto-kinases in resolving blood-clots. The purpose of this study was to investigate the ability of bacterial substances to effectively hydrolyze  $\beta$ -amyloid peptides. Degrading products of several proteinases from *Bacillus pumilus* were evaluated using MALDI-TOF mass-spectrometry, and their toxicity was assessed *in vitro* using cell-culture assays and morphological studies. These enzymes have proved to be non-toxic and were demonstrated to cleave through the functional domains of  $\beta$ -amyloid peptide. By yielding inactive fragments, proteinases of *Bacillus pumilus* may be used as candidate anti-amyloid agents.

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## 1. Introduction

Alzheimer's disease (AD) is a slowly progressive primary neurodegenerative disorder. Its histo-pathological hallmark features, the senile plaques, are extra-cellular deposits of  $\beta$ -amyloid-peptide ( $\beta$ AP) and impaired cellular brain structures associated with inflammatory elements such as microglia. Although a number of hypotheses exist around the patho-mechanism of AD, there is perhaps no single causative agent. It is also dubious if plaques are responsible for and actively involved in the degenerative process, or just innocent by-products formed during the course of the disease that is triggered by yet unknown other factors. According to the amyloid-theory, soluble monomeric  $\beta$ AP is a physiological peptide with several vital functions in the brain (Wu et al., 1995; Schulz, 1996; Huber et al., 1997; Kamenetz et al., 2000), and aggregation is key to its neuro-toxicity (Pike et al., 1993; Dumery et al., 2001), however it is not clearly established what causes polymerization.

Converging lines of evidence suggest that bacterial and viral particles might play a role in cerebral amyloidosis seen in AD, as bacterial endotoxins, among others, have been demonstrated to promote  $\beta$ AP fibrillo-genesis (Reis et al., 2010; Asti and Giorgi, 2014). Neuro-infections may also lead to global cerebral inflammation similar to that is seen in AD brains. In order to facilitate trans-migration of mono-nuclear leukocytes from the periphery into the central nervous system (CNS), inflammatory mediators such as cytokines enhance the permeability of blood-brain barrier (BBB) (de Vries et al., 1996). Similarly, *Helicobacter pylori* also releases products that could induce break-down of the BBB (Kountouras et al., 2014). Other micro-organisms such as *Herpes simplex virus type 1* (HSV1) and *Chlamydia pneumoniae* are postulated to be risk factors for AD (Itzhaki et al., 2004).

In addition to their role in infection, bacteria are also known for their therapeutic effects. Microbial proteinases are widely used as medicinal agents, such as the thrombo-lytic drugs staphylokinase and strepto-kinase. Bacterial proteases have several advantages over similar products of animal origin: they are secreted into the culture medium and can easily undergo purification, they have high stability with low patho-genicity and toxicity, and cultivation of the micro-organisms is also more cost effective. Their role in  $\beta$ -amyloid metabolism, however, is not yet characterized.

$\beta$ AP is continuously produced in low concentrations, and is rapidly turned over in the normal brain: reduced catabolism m

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